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INVITED RESEARCH HIGHLIGHT

Sperm Biology

Looking down on sperm motion: a useful added dimension?

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The assumed association of sperm motility with fertility has long been a preoccupation of clinicians. Whereas assessing fertility of a couple has relatively easy end-points (time to pregnancy, number of children), assessing motility does not. The many methods developed to determine it include assessing a sperm population subjectively (by grading) and objectively (by measuring its motion-induced movement into a light beam by spectrophotometry or nephelometry), and making measurements on individual sperm cells (by stroboscopic or multiple-exposure photography or digitized video-recordings). A new technique reported recently[1] involves holography to determine unrestrained movement patterns of objects in deep chambers over long periods of time. It has been used to provide information on the temporal motility patterns of unrestrained spermatozoa swimming in three-dimensions (hence four-dimensional motility).

Conventional computer-aided sperm analysis (CASA) of spermatozoa is restricted by the requirement that images of the motile object are always in focus;[2] namely, those of a shallow observation chamber (~20 μm) and a short period of observation (~1 s). On the other hand, these limitations do not apply to digital holographic (DH) microscopy, a system of high-resolution possibly lensless imaging that assesses the temporal position of a motile object from the quantitative information contained in holograms. The technique is akin to confocal scanning microscopy, where serial in-focus "optical sections" are reconstructed, but refocusing in DH is by numerical propagation without the necessity for realignment of the optical imaging system. In the double-exposure method, the path of motion is digitally reconstructed from the phase and amplitude of the object's optical wave-front in comparison with that of a reference beam.

In the paper reviewed here,[1] the DH method used for examining spermatozoa was established with suspensions of protozoa, the flagellated *Giardia lamblia* (~8–20 μm) and the ciliated *Paramecium* (~70–140 μm), that bracket the size of a human spermatozoon (~50 μm). The protozoa were placed in 80-μm-deep micro-fluidic chambers (*Giardia*) or cm-deep chambers (*Paramecium*), the light source (a laser beam of 635 nm) was passed through a ground glass screen to reduce "speckle noise" of opalescent solutions, and the software included "proximity correction" to prevent the false tracking of two adjacent motile objects as one, which was further extended to a three-dimensional box to ensure correct tracking of objects moving in different planes. All standard CASA parameters (curvilinear velocity, straight-line velocity, average path velocity, wobble, linearity, straightness index) can be quantified from the data, but along the cell's unrestrained (three-dimensional) track, rather than along a track restrained to the conventional two-dimensional plane.

Despite the ability to detect *Paramecium* through 2 cm of stagnant water, the technique was presumably unable to detect spermatozoa in seminal plasma, because it was removed by density gradient centrifugation. These cells were suspended at 1×10^6 ml^{-1} in Ham's F-10 medium with 6% (*v/v*) CO_2 and analyzed at 37°C. Although the chamber depth was not given, it was deep enough to distinguish sperm tracks within volumes of 100 μm × 100 μm × 60 μm over 11–37 s. It is noteworthy that tracks of five spermatozoa assessed simultaneously were parallel, but whether this is fortuitous, or reflects a limitation of the optical method or fluid drift caused by the edges of the viewing chamber was not commented upon.

Whether this extension of motility assessment to the third physical dimension will bring clinical benefits remains to be proved. It will not replace routine semen analysis, which is rapidly performed on neat semen samples. Within the female tract the fluid environment and volume which spermatozoa encounter may enforce flagellar motions distinct from those quantified in an experimental unobstructed three-dimensional space. Upon ejaculation, spermatozoa within liquefying seminal in the narrow confines of the cervix migrate out of it into cervical mucus, both fluids of different and changing osmolalities[3] that are very different from that of protein-free Ham's medium. The utero-tubal junction is less restrictive to motion than the cervix, and its fluid composition is partially known, but the geometry of the space and the motion of this organ around the time of intercourse are unknown.

Such realities hold equally for the relevance of conventional CASA measurements on two-dimensional motion, so whether the novel four-dimensional analysis is of more clinical relevance will be shown by analysis of the data generated from samples of known fertility. Its use will be vindicated if these data reveal that the presence or size of sub-populations of spermatozoa exhibiting particular three-dimensional motions (such as a spiralling descent), which are detectable only by the four-dimensional method, is predictive of some measure of fertility determined on the same samples used for intrauterine insemination or *in vitro* fertilization.

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